

In pursuit of the “holy grail”: recombinant allergens and peptides as catalysts for the allergen-specific immunotherapy

Mohapatra S, Cao Y, Ni H, Salo D. In pursuit of the “holy grail”: recombinant allergens and peptides as catalysts for the allergen-specific immunotherapy.

Allergy 1995; 50 (suppl 25): 37–44. © Munksgaard 1995.

This presentation reviews recent progress in knowledge of (i) molecular biology and immunology of allergens and (ii) the methods for the modulation of allergen-specific immune response, with special emphasis on our grass pollen allergens, particularly *Poa p* IX AL, used as a model. The last few years have seen tremendous progress in molecular characterization of allergens, leading to synthesis of a number of recombinant allergens. Furthermore, studies of the structure-activity relationship of allergens have led to the delineation of the epitopes of these allergens and of widespread cross-reactivities among diverse allergens. In view of the requirement for extensive pre-clinical studies, the application of the recombinant allergens and epitopes in the clinical realm has just begun. Studies in murine systems suggest that these allergens and/or their epitopic peptides downregulate allergen-specific immune responses *de novo*. The potential and drawbacks of the recombinant allergens and peptides for improving the existing immunotherapeutic approaches and for developing new approaches to specific immunotherapy are discussed.

S. Mohapatra, Y. Cao, H. Ni, D. Salo

Department of Immunology,
University of Manitoba, Winnipeg,
Manitoba, Canada

Key words: immunotherapy; recombinant allergen; peptide immunotherapy; TCR peptide

Shyam S. Mohapatra
Department of Immunology,
University of Manitoba,
Winnipeg, Manitoba,
Canada R3T 2N2

Since its introduction in the early 1900s, allergen injection immunotherapy (AIIT) has been the “holy grail” of clinical allergology and has been practiced as the one and only immunologically specific therapy against allergic rhinitis and asthma. AIIT involves repeated injections of the appropriate allergen (AL) or allergens over prolonged periods. Although the molecular and cellular mechanism of AIIT is not fully understood, the effectiveness of this therapy in the hands of expert allergists has been demonstrated (5, 40). As currently practiced, AIIT, in addition to being cumbersome, expensive and associated with the risk of fatal side reactions, including systematic anaphylaxis, often leads to no improvement of the patient's condition. This lack of predictable efficacy is due to the fact that the available commercial preparations consist of heterogeneous (crude) extracts of the corresponding pollen(s) and may contain the relevant ALs in only minute and irreproducible amounts, leading up to 100-fold variation in allergenic potency. Also the extracts often contain constituents unrelated to the few ALs, to which the patient is actually allergic; clearly the former com-

ponents may lead to the induction of immunoglobulin E (IgE) antibodies (ABs) with new specificities (15). The main drawbacks that limit the efficacy and use of AIIT are (i) most importantly, the lack of sufficient amounts of pure and standardized preparations of ALs and (ii) lack of knowledge of the precise structural relationships among the ALs. To circumvent these limitations, recently several laboratories have employed recombinant DNA (rDNA) procedures for the molecular characterization of major ALs of mites, hornets and pollens of trees, grasses and weeds, etc. (27, 28). Indeed, the last five years have seen spectacular advances in the field of AL characterization: the advent of the recombinant allergens (rALs), in particular, has played a significant role in furthering our knowledge of the structure-function relationships of ALs and understanding of the AL-induced IgE-mediated allergies. In this report we review the recent advantages in the field of molecular biology of allergens and how this may aid in renovating the currently practiced AIIT, with special emphasis on progress from our laboratory on recombinant grass pollen allergens.

Immunobiology of the allergic response: a bird's eye view

T cells and T cell cytokines play a pivotal role on the induction and persistence of IgE Abs and influence immune response at the level of recognition of the ALs (43). Antigen-presenting cells (APC) process the incoming ALs to their constituent peptidic fragments and then present these to T cell receptors (TCR) in the context of an appropriate molecule of major histocompatibility complex (MHC). The "specificity" of the humoral immune response to ALs (as all other antigens) is governed by the trimolecular (MHC-peptide-TCR) interaction, which constitutes the very first step in the initiation of immune response (8). However, engagement of TCR alone is not sufficient to generate Ab response; it requires a second co-stimulatory signal provided by the interaction of B7 molecule on a professional APC and CD28 on the T cells (16, 20). Moreover, mere engagement of TCR in the absence of a second signal leads to the T cell anergy (17).

Activated T cells produce "help" for IgE synthesis by releasing lymphokines interleukin 4 (IL-4). Of late, a large body of evidence in both humans and animal systems suggests a preponderance of type 2 helper T (Th2) cell responses in allergic disorders (35, 43). Th2 cells produce cytokines such as IL-4, IL-5, IL-10, as opposed to the "protective" Th1 type of cells which produce IL-2 and interferon γ (IFN- γ). The ratio of the major IgE-switch factor IL-4 and its regulator IFN- γ determines the level of IgE synthesis; these two cytokines also mediate the differentiation of Th2 and Th1 subsets, respectively. It should be possible to exploit the reciprocal regulation of Th1 and Th2 to develop methods for suppression of IgE AB production; however, cytokines *per se* may be inadequate, since their therapeutic effects are not specific and not always predictable, as (i) IL-4 is also released by mast cells and basophil-like cells and CD8⁺ or CD4⁺CD8⁻ T cells (43, 45); and (ii) IL-4 activity is affected by the local milieu of soluble receptors and other cytokines such as IL-10, IL-12 and IL-13, which are also involved in Th1-Th2 regulation (13, 43).

Molecular biology and immunology of allergens

Molecular cloning and sequencing of allergens

Molecular cloning of ALs has led to the determination of the primary structure of numerous ALs. To date, a majority of the allergens have been cloned in the phage expression vectors λ gt 11. A list of allergens that have been molecularly cloned and sequenced using this strategy has been published recently (27, 28). Thus, about 50 of the major allergens

have been cloned; this number has tripled since 1992, and 5 to 10 new allergenic proteins are being added to this list every year. In addition to the cloned allergens, a number of other allergens have been reported (27), and presumably the cloning of these allergens is currently in progress.

In grass pollens, in particular, two major groups of proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) molecular weights in the range of 28–33 and 35–37 kDa have been reported that were cloned and characterized (28, 29, 36, 39). Previously, research in this laboratory led to the (i) determination of the primary structures of the 3 full-length types of complementary DNA (cDNA), i.e., KBG31, KBG60 and KBG41, encoding the *Poa* oIX group of ALs with molecular weights in the range of 28–33 kDa, (ii) cloning in *Escherichia coli* of a number of overlapping cDNAs in expression vectors, pWR590 (45) and pGEX-2T-1 (37) series of plasmids, which allowed synthesis in milligram amounts of corresponding rALs, and (iii) immunologic studies of these rALs *vis-à-vis* native ALs utilizing sera of grass pollen allergic patients, which revealed that (a) *Poa* pIX group of ALs comprises at least 5 basic components (15), (b) about 51% of 1112 individual sera from 15 different countries obtained as a result of an international collaborative study of allergic patients reacted with recombinant KBG8.3, (c) a strong positive correlation ($r = 0.90$) was observed between the levels of IgE antibodies reacting with rAL and with the native proteins extracted from KBG pollen (55) and (d) the pollens of 10 other grasses were shown to contain ALs homologous to *Poa* pIX, as indicated by their reactivity with anti-rKBG8.3 Abs (56).

More recently, a partial cDNA clone, 51, which reacted with sera of patients allergic to grass pollen, was characterized (23). Nucleotide sequence analysis of clone 51 indicates that the polypeptide encoded by this cDNA is different from that of the known recombinant grass pollen ALs. Using the murine antiserum to this rAL, produced with the aid of the pGEX-2T-1 expression system (37), a predominant polypeptide of about 58 kDa in size was detectable in SDS-PAGE immunoblot analysis of KBG pollen proteins (Fig. 1). Most importantly, (i) the reactivity of this antiserum, with a number of polypeptides, which ranged in size from 30 to 115 kDa, from pollen extracts of several grasses, birch, ragweed and parietaria, and (ii) the reverse transcription hybridization, polymerase chain reaction (RT-PCR) products from various pollens with radiolabeled 51-cDNA, demonstrated the cross-reactivity (CR) of this AL with other pollen ALs. Analysis by enzyme-linked immunosorbent assay (ELISA) using sera of about 1000 individuals allergic to pollens demonstrated that about 40% of these

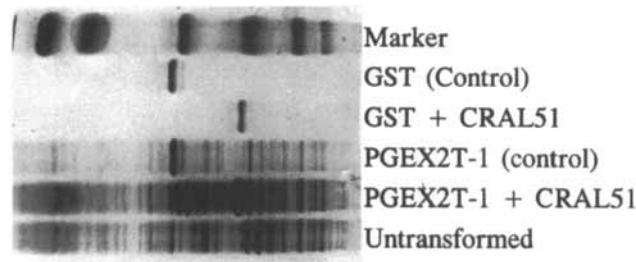


Fig. 1. **Top.** Expression cloning of CRAL 51 cDNA. The cDNA insert was ligated to the pGEX 2T-1 vector and transformed into *E. coli*. The transformants were screened for the production of recombinant fusion protein. **Bottom.** An antiserum raised against this fusion protein reacted predominantly with a 58-kDa polypeptide on Western blot of KBG pollen extract.

sera possessed IgE Abs specific to KBG extract and about 40% of these sera possessed antibodies specific to recombinant KBG51 protein. On the basis of these findings, it is inferred that the cloned AL represents a member of family of high cross-reactive ALs in plant pollens.

Synthesis of rALs

The major goal of cloning allergens is to produce the recombinant proteins in unlimited amounts for use in basic as well as clinical studies. To date, only a few of these allergens have been cloned in plasmid vectors for high-level expression in *E. coli*, which allow synthesis of the allergenic protein either (i) as a fusion protein, i.e., linked to β -galactosidase (46) or glutathione S-transferase (37), or (ii) as rALs by itself utilizing vectors such as pKK233 series of expression plasmids (6). Recently, both yeast (9) and baculovirus (49) expression systems have been utilized for the synthesis of certain rALs. Recent studies in our laboratory have shown that the baculovirus expression system provides a more efficient expression system not only in terms of the amount of rAL produced but also IgE binding of these rAL (data not shown); the latter is presumably due to glycosylation of the recombinant proteins, which allows appropriate folding similar to that of the native protein.

One of the concerns with the synthesis of rALs is whether the rAL in question is functionally comparable to its native counterpart from the corresponding source. Several of the rALs have been shown to react with IgE antibodies specific for their native counterparts (21, 55). Further exploitation of yeast and baculovirus expression systems is expected to lead to the production of the rALs that, when produced in *E. coli*, do not bind IgE efficiently. Production of these rALs in virtually unlimited quantities is expected to lead to the availability of standardized allergens (30) for improved diagnosis (38, 50) and therapy of specific allergies.

Analysis of B- and T-cell epitopes

Utilizing overlapping peptides produced by recombinant DNA methods, i.e., by expression cloning of overlapping cDNAs and the synthesis of the corresponding peptides in *E. coli*, and overlapping peptides chemically synthesized by automated methods or by the manual methods such as, using the polypropylene pins, the B cell epitopes for the *Poa pIX* allergen were examined (58). The results to date revealed that *Poa pIX* ALs contain multiple epitopes (Fig. 2). A number of these epitopes coincide with the human IgE binding epitopes. Nevertheless, such peptides permit the detection of linear epitopes and not of the conformational epitopes. The T-cell epitopes of this allergen were also examined by T-cell proliferation assay using peripheral blood monocytes (PBMC) and/or T-cell lines and clones. From the schematic diagram shown in Fig. 1, it is concluded that these grass allergens have a number of epitopes and that the B- and T-cell epitopes appeared to overlap. Moreover, the epitopes recognized by individuals differed.

Knowledge of the B- and T-cell epitopes is mandatory for the formulation of any therapeutic modality(ies) based on epitopes. Therefore, similar to that of the *Poa pIX* ALs, the availability of structural information has prompted studies on delineation of B (3, 14, 42, 48) and T-cell epitopes (11, 18, 19, 47) of a number of other major ALs of mites, cat and pollens of trees, ragweed and grasses etc., utilizing both human and model animal systems. However, determination of the complete repertoire of epitopes of diverse environmental aeroallergens remains one of the most arduous and formidable tasks. Particularly since several of the major high-molecular weight (> 5-kDa) allergens contain multiple epitopes, and the epitopes recognized by different individuals may vary.

MAPPING OF EPITOPES OF rKBG60 ALLERGEN USING OVERLAPPING PEPTIDES



Fig. 2. Schematic diagram showing epitopes of rKBG60 allergen using a set of overlapping peptides. The peptides recognized by the human IgE antibodies and human PBMC, T-cell lines and clones are shown. The approximate locations and amino acid sequences of the peptides are shadowed.

Availability of rALs and the knowledge of their primary structures and B- and T-cell epitopes led to the elucidation of cross-reactivities among different allergens, because of obvious implications in therapy and diagnosis. Cross-reactivities have been studied both at the B- and T-cell level not only among allergens belonging to the same aeroallergen source but also among different aeroallergens (4, 28, 31, 32). In principle, the same methods as outlined previously for the identification of B- and T-cell reactivities have been used for the delineation of cross-reactivities among allergens and epitopes. Sequence homology among various allergens and their epitopes appears to be a principal reason for the extensive cross-reactivities observed among allergens. In addition, a method for the analysis of structural motifs within and among diverse groups of aeroallergens was recently suggested as an approach to identify the major IgE-binding motifs on allergens (32). However, whether the motifs predicted by this method would stand functional scrutiny remains to be elucidated.

Allergen-specific immunotherapy: fact or fancy?

As stated previously, all of the presently available therapies have the common disadvantage of utilizing ill-defined allergenic extracts(s), although some purified allergens have been used in experimental studies and have been found to be effective. Aqueous and formaldehyde-polymerized components of allergenic extracts and allergenic components adsorbed onto calcium phosphate or aluminum hydroxide are being used for the currently practiced AIIT. In addition, the efficacies of some other forms of immunotherapy, involving injection of allergenic extracts sublingually,

orally or as encapsulated ingredients of liposomes, are being tested in clinical trials. However, since the rALs have only synthesized relatively recently, they have not yet been utilized for clinical studies. These products have to undergo rigorous testing by the regulatory agencies prior to their approval for such studies. A number of approaches that may be potentially useful for the application of the RAs for therapeutic purposes are schematically shown in Fig. 3. These methods include both allergen- and epitope (peptide)-based methods.

Therapy with recombinant allergens

The advent of rALs makes it possible to identify the unique protein(s) to which the individual is sensitized; hence, it may be envisioned that future immunotherapy will be based on the use of selective allergen(s). To examine the efficacy of allergen-specific immunotherapy, we exploited a murine model of allergy developed specifically for the *Poa p*-IX rALs. Thus, groups of B6D3F1 mice were pretreated i.v. with soluble rKBG8.3 AL and the immunized with the same AL in alum; the specific IgE Ab production was examined. The rKBG8.3 specific IgE Ab titers were reduced (>90%) in treated mice as opposed to that of control mice, suggesting that pretreatment induced AL-specific tolerance (data not shown). The tolerance lasted for a period of about 85 days (as long as was examined). Immunization with KBG extract of mice pretreated with RA led to marginal decline in IgE titers, but the IgG2a titers were increased. These results suggest the potential of RA for specific immunotherapy of allergic conditions.

However, in case of humans, because of the purity of rAL preparations, their anaphylactogenic index may be extremely high even at very low doses, and extreme care may be required when they are administered by allergy specialists. Moreover, enteric-coated (53) or liposome-packed (2) rALs may also be useful for immunotherapy.

Therapy with r-allergoids

In addition to use of aqueous RAs, it may be envisaged that a major improvement in allergen-specific immunotherapy may be achieved by the utilization of chemically modified rALs, which should render these regimens essentially non-allergenic, while retaining the antigenicity. Thus, approaches involving allergoids produced by formaldehyde polymerization (24) of rALs, the "r-allergoids", appear to be promising for immunotherapy. Also pegylation of rALs by monomethoxypolyethylene glycol (10) or conjugation with *N*-formyl-methionyl-leucyl-phenylalanine (54) may lead to therapeutic derivatives of these al-

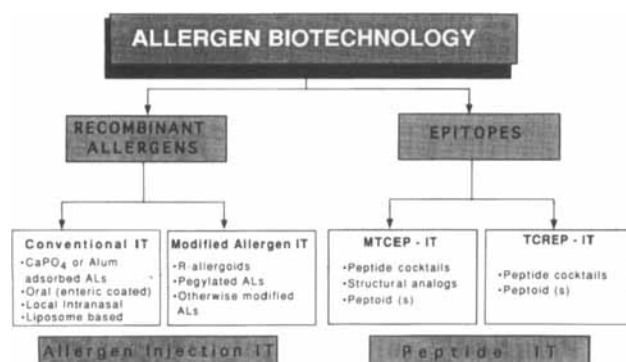


Fig. 3. Potential application of recombinant allergens and epitopes. The allergen-based therapies may involve conventional immunotherapy as well as therapy with modified allergens; peptide(s) constituting the minimal T cell epitopes of the allergens *per se* or the peptides derived from their corresponding T cell receptors. The boxed text show the examples of some possible approaches.

lergens. On the basis of these, it is reasonable to predict that, in contrast to conventional immunotherapy, the use of relatively fewer injections of these modified rALs would lead to safer and more efficacious therapies.

Novel approaches to specific therapies

Several experimental approaches to therapy have been reported recently. One such approach involved treatment of allergic rhinitis and asthma with allergen-antibody complexes (25). The second approach may comprise injection of rALs in conjunction with adjuvants other than alum, which not only enhance the immune responsiveness but may also divert the responses toward a Th1 type, as shown in our recent studies with the rKBG8.3 AL linked to another antigen β -galactosidase (Fig. 4) (57). The third possible approach has emerged from our recent *in vitro* studies, in collaboration with S. Romagnani's laboratory, utilizing human T cell clones. It was thus shown that the culture of T cell lines from atopic individuals with one RA of *Poa pIX* family of allergens and IFN- α led to the development of "protective", i.e., Th1 cells instead of the "pathogenic", i.e., Th2 cells. More recently, IL-12 has been reported to act as adjuvant leading to preferential induction of Th1 type of response (1). Thus, administration of rALs in conjunction with cytokines may aid in di-

verging allergen-specific immune responses from IgE to IgG formation.

Peptide-based therapies: will they really work?

Advances of our knowledge of cellular and molecular factors determining IgE-mediated allergies, in conjunction with the definition of increasing numbers of epitopes of allergens, provide a sound basis for the development of new approaches for therapy of allergies using peptide epitopes. Since T cells play a dominant role in IgE synthesis, a number of strategies have been proposed that are directed at anergizing these cells and/or at interfering with the formation of trimolecular complexes involving the interaction of the MHC-peptide duplex with the appropriate Th cell receptor (27). Whether treatment with peptides would be a feasible alternative to existing immunotherapy remains to be elucidated. A brief overview and a critical appraisal for the potential of treatment of allergic disorders with peptides is provided below.

Treatment with allergen-peptides and peptoids

A strategy for modulating immune responses involved treatment with the peptidic fragments of the desired allergens produced by proteinase digestion, as was shown with ragweed allergen *Amb a I* (22, 26). Although the precise mechanism underlying this approach is not known, the treatment reduced clinical symptom scores and was considered to be successful, the study involved only a few individuals and was incomplete. A number of studies have also addressed the use of defined minimal T-cells peptides for treatment of allergies since: (i) TCR-peptide ligation plays a critical role in differentiation of T cells to Th1 or Th2 type (12), and it is possible to manipulate this interaction in favor of the desired (protective) response; and (ii) the AL-peptides may enable induction of peripheral T-cell tolerance as has been achieved recently in experimental animal by injection with T-cell peptides of major cat allergen *Fel d I* (7).

In similar studies, in our laboratory, we examined the effect of peptide treatment on subsequent IgE antibody production. Thus, mice were treated with peptides and later immunized with the allergen rKBG8.3 (Fig. 5). The IgE titers examined on day 14 and day 30 demonstrated that the titers of mice treated with either of the selected peptides #23 and #26 were reduced in comparison with that of the control. Moreover, the T cells of mice that had been treated with T-cell epitopic peptides were found to possess a lower proliferative capacity with respect to the epitopes used for pre-incubation; however, the

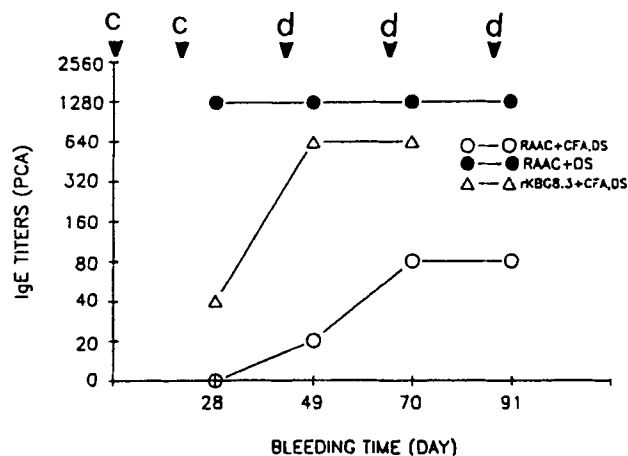
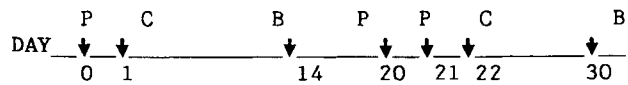


Fig. 4. Modulation of AL-specific IgE antibody production treatment with RAAC protein and CFA as adjuvant. Three groups of mice used in this study were immunized on day 1, 21, 42, 63 and 84 (marked by arrowheads) with appropriate antigen and adjuvant as follows. First group (control) immunized with RAAC protein with dextran sulphate (DS) as adjuvant. The second group were immunized with rKBG8.3 with CFA as adjuvant on day 1 and 21 and DS as adjuvant on day 42, 63. The third group were immunized with RAAC protein with CFA as adjuvant on day 1 and 21 and DS as adjuvant 42, 63 and 84. The IgE antibody titers were determined in pooled sera on day 28, 49, 70 and 91 by PCA assay (adapted from Zhang & Mohapatra, J Immunol 1993; 151: 791).

Protocol:



PCA TITERS

		Primary			Secondary	
	C	#23	#26	C	#23	#26
8.3	80	nil	nil	1280	640	320
KBG	40	nil	nil	640	320	320

Fig. 5. Suppression of IgE immune responses by T cell of *Poa p*-IX allergen, rKBG8.3. Upper panel: an outline of the protocol; groups of four mice each were treated with either the peptide (P) (300 μ g/mice) or PBS (control) and subsequently immunized with rKBG8.3 (C). The mice were bled (B) for antibody assay on days shown. Lower panel: The PCA titers of mice treated with peptides and PBS are shown. Either rKBG8.3 or KBG was used as the challenge antigen.

proliferative capacity of these T cells to unrelated epitopes of the corresponding RA was only slightly reduced (Mohapatra, unpublished results). These studies clearly demonstrate the potential of peptide treatment in downregulation of IgE antibody responses. Nevertheless, given that some of the major allergens, particularly pollen allergens, contain a number of T cell epitopes and that allergic individuals differ with respect to their recognition of these epitopes, the success of this approach in presensitized individuals is difficult to predict at this time.

It is noteworthy that peptides used for treatment may be modified with respect to their structure on the basis of the knowledge of the core residues involved in interactions with TCR. In principle, certain structural analogs of these peptides that do not cause cross-linking, i.e., devoid of B-cell binding activity and that engage the TCR but do not stimulate or, if then stimulate, do not lead to IL-4 production, may be found (12). Furthermore, the "peptoids", i.e., peptides with appropriate chemical modification, may lead to induction of antigen-specific tolerance. Obviously, all approaches targeted via trimolecular interaction are MHC-restricted and will only be effective if there is considerable immunodominance for the antigen in question.

Treatment with TCR peptides of AL-specific T cells

As stated above, TCR plays a pivotal role in antigen recognition. A number of studies in autoimmunity have revealed that the TCR gene usage is restricted, which led to the formulation of the TCR peptide-

based antigen-specific therapy (51). To date, TCR V gene analysis has been performed only for the dust mite and grass AL-specific T cell clones (32, 52). The results of mite studies indicate a limited usage of TCR V β genes, i.e., V β 3 or V β 6, which recognize the main mite allergens, *Der p*I; similarly, a limited usage of V α genes was demonstrated in T-cell clones specific for grass ALs. In addition, analysis of TCRs in a complex leishmaniasis also reveals the expansion of a TCR-restricted population of T cells (40). It is noteworthy that in all of these studies immunodominance (34) with respect to TCR gene usage was observed. These studies have led to formulation of peptide immunotherapy approach involving TCR peptides. The advantage of these peptides is that they are not related or may not share the structure of the allergen; therefore, they may not induce any adverse reactions.

To this end, in a recent study, downregulation of specific antibody responses to egg allergen, ovalbumin (OVA), was achieved in mice by treatment with synthetic peptides corresponding to the CDR3 region(s) of the α and β chains of T-cell receptors (TCR) of cloned OVA-specific CD8⁺ regulatory cells (33). Thus, pretreatment of mice with these peptides led to persistent downregulation (>200 days) of the potential of these mice to produce anti-OVA IgG1 and IgE antibodies. More importantly, established OVA-specific antibody production was markedly reduced following peptide treatment. The injection of these TCR peptide(s) led to the induction of CD4⁺ T cells, which regulated the immune response in an antigen-specific manner. These findings demonstrate the potential of treatment with TCR peptides of antigen-specific regulatory CD8⁺ T cells for the downregulation of unwanted antibody responses. The mechanism underlying this phenomenon remains to be elucidated.

Concluding remarks

Despite the progress in knowledge of molecular immunologic properties of allergens, the application of this knowledge in the clinical arena is at infancy. However, synthesis of certain major recombinant allergens by fermentation and allied technologies, as reported recently, is anticipated to expedite the use of these allergens for therapeutic studies. Furthermore, the knowledge of epitopes of ALs has advanced to a point whereby some of these epitopes are in clinical trial. At present, one can only be optimistic about the application of these recent advances toward either revival of the existing immunotherapy or developing certain new immunotherapeutic procedures based on the rational immunologic principles. Taking into account the complexity of

allergic conditions, it is naive to expect that one form of AL-specific immunotherapy would be effective in treatment of allergies. For certain allergies (such as ragweed or grass?), protocols would involve a battery of ALs to target the polyclonal populations of T cells, thereby increasing the likelihood of successful treatment. Also, it is more likely that different forms of this type of immunotherapy used in concert or with other therapeutic agents may prove to be generally effective, particularly in chronic allergic conditions. As we continue to learn in greater detail the mechanisms involved in establishment of allergic conditions, we may be able to devise additional and more effective protocols for treatment.

Acknowledgements

I gratefully acknowledge the efforts and enthusiasm of my previous associates Drs. Egil Olsen, Lei Zhang, Min Yang, Yan Yun Wang, Subhra Mohapatra and Andre Silvanovich, whose initial contributions have been instrumental in these studies. I also thank Professors A. Sehon, Sergio Romagnani and David Marsh for collaborative support. This work was supported by grants from the Medical Research Council of Canada, the Manitoba Health Research Council of Canada, Manitoba Medical Services Foundation, Winnipeg, and Connaught Laboratories Limited, Toronto, Ontario.

References

1. AFONSO LLCC, SCHARTON TM, VIEIRA LQ et al. The adjuvant effect of IL-12 in a vaccine against *Leishmania major*. *Science* 1994; 263: 235.
2. ARORA N, FANGAL SV. Efficacy of liposome entrapped allergen in downregulation of IgE response in mice. *Clin Exp Allergy* 1992; 22: 35-42.
3. ATASSI H, ATASSI MZ. Antibody recognition of ragweed allergen Ra3: localization of the full profile of the continuous antigenic sites by synthetic overlapping peptides representing the entire protein chain. *Eur J Immunol* 1986; 16: 229.
4. BASKAR S, PARRONCHI P, MOHAPATRA SS, ROMAGNANI S, ANSARI AA. Human T cell responses to the purified pollen allergens of the grass, *Lolium perenne*: relationship between structural homology and T cell recognition. *J Immunol* 1992; 148: 2378-2383.
5. BIYSQYET, J, MICHEL FB. Advances in specific immunotherapy. *Clin Exp Allergy* 1992; 22: 889.
6. BREITENEDER N, PETTENBURGER H, BITO A et al. The gene coding for the major birch pollen allergen, Bet v I is highly homologous to a peak diseases resistance gene. *EMBO J* 1989; 8: 1935-8.
7. BRINER T, KUO M-C, KEATING KM, ROGERS BL, GREENSTEIN J. Peripheral T cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. *Proc Natl Acad Sci U S A* 1993; 90: 7608-7612.
8. BUUS S, SETTE A, COLON S et al. *Science* 1987; 235: 1353.
9. CHUA KY, KEHAL PK, THOMAS WR et al. High frequency binding of IgE to the Der P allergen expressed in yeast. *J Allergy Clin Immunol* 1992; 89: 95-10.
10. DREBORG S, AKERBLOM EB. Immunotherapy with monomethoxy polyethylene glycol modified allergens. *Crit Rev Ther Drug Carrier Syst* 1990; 6: 315-365.
11. EBNER C, SZEPPALUSI Z, FERREIRA F et al. Identification of multiple T cell epitopes on Bet V I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993; 150: 1047.
12. EVAVOLD BD, WILLIAMS SG, HSU BL et al. Complete dissection of the Hb (64-76) determinant using T helper 1 and T helper clones and T cell hybridomas. *J Immunol* 1992; 148: 347.
13. FITCH FW, MCKISIC MD, LANCKI DW et al. Differential regulation of murine T lymphocyte subsets. *Annu Rev Immunol* 1993; 11: 29.
14. HAMILTON RG. Allergy testing. *Curr Opin Immunol* 1990; 2: 5513.
15. JENKINS MK, SWARTCHZ RH. *J Exp Med* 182: 165: 302.
16. JENKINS MK, TAYLOR PS, NORTON SD et al. J CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *Immunology* 1991; 147: 2461.
17. JOOST VAN NEERVAN RJ, VAN DE POL MM, VAN MILLIGEN FJ et al. Characterization of cat dander-specific T lymphocytes from atopic patients. *J Immunol* 1994; 152: 4203.
18. GREENE WK, CYSTER JG, CHA KY et al. IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen Der p I. *J Immunol* 1991; 147: 3769.
19. KURISAKI JI, ATASSI H, ATASSI MZ. T cell recognition of ragweed allergen Ra3: localization of the full T cell recognition profile by synthetic overlapping peptides representing the entire protein chain. *Eur J Immunol* 1986; 16: 236.
20. LANZAVECCHIA A. Strategies for immune intervention. *Science* 1983; 260: 937.
21. LARSEN JN, CASALS AB, FROM NB, et al. Characterization of recombinant Bet VI, the major allergen of *Betula verrucosa* (white birch), produced by fed-batch fermentation. *Int Arch Allergy Immunol* 1993; 102: 249-258.
22. LITWIN A, PESCE AJ, FISCHER T, MICHAEL JG. Regulation of the human immune responses to ragweed pollen by immunotherapy. A control trial comparing the effect of immunosuppressive peptidic fragments of short ragweed with standard treatment. *Clin Exp Allergy* 1991; 21: 457-465.
23. LUO M, NI H, SALO D, MOHAPATRA SS. Identification of a novel highly crossreactive allergen of plant pollens. *J Allergy Clin Immunol* 1994; 93: 252.
24. MAASCH HJ, MARSH DG. Standardized extracts modified allergen-allergoids. *Clin Rev Allergy* 1987; 5: 89.
25. MACHIELS JJ, SOMVILLE MA, JACQUEMIN MG, SAINT-REMY JM. Allergen-antibody complexes can efficiently prevent seasonal rhinitis and asthma in grass pollen hypersensitive patients: allergen-antibody complex immunotherapy. *Allergy* 1991; 46: 335-348.
26. MICHAEL JG, LITWIN A, HASSERT V, PESCE A. Modulation of the immune response to ragweed allergens by peptidic fragments. *Clin Exp Allergy* 1990; 20: 669-674.
27. MOHAPATRA SS. Modulation of allergen-specific antibody responses by T cell-based peptide vaccines: principles and potential. *Clin Rev Allergy* 1994; 12: 3-22.
28. MOHAPATRA SS. Diagnosis and therapy of IgE mediated allergies with recombinant allergens: *Poa pratensis* IV as a model. *Pharmacia Allergy Res Found Awards Book* 1992: 4-15.
29. MOHAPATRA SS. Molecular characterization of pollen allergens: implications for immunotherapy? In: AGASHE SN, ed. Recent trends in aerobiology, allergy and immunology. New Delhi: Oxford IBH, 1994: 278-292.
30. MOHAPATRA SS. Recombinant allergens and allergen standardization. *J Allergy Clin Immunol* 1992; 89: 921-922.
31. MOHAPATRA SS. Analysis of structural motifs of allergenic proteins as a tool in studies of cross-reactivity among allergens. In: KRAFT D, SEHON AH, ed. Molecular biology and immunology of allergens. Boca Raton: CRC Press, 1993: 69-75.

32. MOHAPATRA SS, MOHAPATRA S, YANG M et al. Molecular basis of cross-reactivity among allergen-specific human T cells: T cell receptor gene usage and epitope structure. *Immunology* 1994; 81: 15–20.
33. MOHAPATRA S, CHEN T, MASARU T et al. Analysis of T cell receptor $\alpha\beta$ chains of CD8⁺ suppressor T cells induced by tolerogenic conjugates of antigen and mPEG: involvement of TCR α CDR3 domain in immunosuppression. *J Immunol* 1993; 151: 688.
34. MOHAPATRA SS. Determinant spreading: implications in vaccine design of atopic disorders. *Immunol Today* 1994; 15: 596–597.
35. MOSMANN TR, CHERWINSKY H, BOND MW et al. Two types of murine helper T cell clones. I. Definition according to profile of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348.
36. OLSEN E, MOHAPATRA SS. Expression and thrombin cleavage of recombinant proteins fused to glutathione S-transferase. *Int Arch Allergy Immunol* 1992; 98: 343–348.
37. OLSEN E, ZHANG L, HILL RD et al. Identification and characterization of the *Poa pIX* group of basic allergens of Kentucky bluegrass pollen. *J Immunol* 1991; 147: 205–211.
38. OLSEN E, MOHAPATRA SS. Recombinant allergens and diagnosis of specific allergies. *Ann Allergy* 1994; 72: 499–506.
39. PEREZ M, ISHIOKA GV, WALKER LE et al. cDNA cloning and immunological characterization of the ryegrass allergen *Lol pI*. *J Biol Chem* 1990; 265: 16210–16216.
40. Position paper on allergen immunotherapy. *Clin Exp Allergy* 1993; 23: 1–44.
41. REINER SL, WANG Z-E, HATAM F et al. Th1 and Th2 cell antigen receptors in experimental leishmaniasis. *Science* 1993; 259: 1457.
42. ROGERS BL, MORGENSTERN JP, GARMAN RD et al. Recombinant Fel d I: expression, purification, IgE binding and reaction with cat allergic human T cells. *Mol Immunol* 1993; 30: 559.
43. ROMAGNANI S. Induction of TH1 and TH2 responses, a key role for the natural immune responses? *Immunol Today* 1992; 13: 379.
44. SALGAME P, YAMAMURA M, BLOOM BR, MODLIN RL. *Chem Immunol* 1992; 54: 44–59.
45. SALGAME P, YAMAMURA M, BLOOM BR, MODLIN RL. *Annu Rev Immunol* 1993; 11: 29.
46. SILVANOVITCH A, ASTWOOD J, ZHANG L et al. Nucleotide sequence analysis of three *Poa pIX* isoallergens of Kentucky bluegrass pollen. *J Biol Chem* 1991; 266: 1204–1210.
47. SPIEGELBERG HL, BECK L, STEVENSON DD, ISHIOKA GY. Recognition of T cell epitopes and lymphokine secretion by ryegrass allergen *Lolium perenne*-I specific human T cell clones. *J Immunol* 1994; 152: 4706–4711.
48. SUPHIOGLU C, SINGH MB, KNOW RB. Peptide mapping analysis of group I allergens of grass pollens. *Int Arch Allergy Immunol* 1993; 102: 144–151.
49. TOMALSKI MD, KING, TP, MILLER LK. Expression of hornet genes encoding venom allergen antigen 5 in insects. *Arch Insect Biochem Physiol* 1993; 22: 303–313.
50. VALENTA R, VRTALA S, EBNER C et al. Diagnosis of grass pollen allergy with recombinant timothy grass pollen allergens. *Int Arch Allergy Immunol* 1992; 97: 287–294.
51. VANDENBARK AA, CHOU YK, BOURDETTE DN et al. T cell receptor peptide therapy for autoimmune disease. *J Autoimmun* 1992; 5: 83.
52. WEDDERBURN LR, O'HEHIR RE, HEWITT CRA et al. *In vivo* clonal dominance and limited TCR gene usage in human CD4⁺ T cell recognition of house dust mite allergens. *Proc Natl Acad Sci U S A* 1993; 90: 8214.
53. WHEELER AW, HENDERSON DC, YOULTEN LJ et al. Immunogenicity in guinea pigs and tolerance in grass pollen-sensitive volunteers of enteric coated grass pollen allergens. *Int Arch Allergy Appl Immunol* 1987; 83: 354–358.
54. WHEELER AW, WHITTALL N, COOK RM, SPACKMAN VM, MORAN DM. T cell reactivity of conjugates of *N*-formyl-methionyl-leucyl-phenylalanine and rye grass pollen allergens. *Int Arch Allergy Immunol* 1987; 84: 69–73.
55. YANG M, OLSEN E, DOLOVICH J, SEHON A, MOHAPATRA SS. Immunologic characterization of a recombinant Kentucky bluegrass (*Poa pratensis*) allergenic peptide. *J Allergy Clin Immunol* 1991; 87: 1096–1104.
56. ZHANG L, KISIL FT, SEHON AH, MOHAPATRA SS. Allergenic and antigenic cross-reactivities of group IX grass pollen allergens. *Int Arch Allergy Appl Immunol* 1991; 96: 28–33.
57. ZHANG L, MOHAPATRA SS. Antigen- and isotype-specific immune responses to recombinant antigen-allergen chimeric (RAAC) protein. *J Immunol* 1993; 151: 791.
58. ZHANG L, OLSEN E, KISIL FT, HILL RD, SEHON AH, MOHAPATRA SS. Mapping of antibody binding epitopes of a recombinant *Poa pIX* allergen. *Mol Immunol* 1992; 29: 1383–1389.